

Physical Therapy for Type III Spinal Muscular Atrophy in a Uyghur Pedigree

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Research

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Abstract

Background: Spinal muscular atrophy (SMA) is a genetically determined neuromuscular disease with predominantly proximal muscle atrophy and weakness caused by degeneration of lower motor neurons in the central nervous system. SMN1 is recognized as an SMA causing gene. The SMN2 copy numbers was assessed for SMA severity. Multiple ligation-dependent probe amplification (MLPA) technique allows to confirm the diagnosis of SMA. The clinical spectrum in affected individuals varies widely from severe generalized weakness (SMA types I and II) to modest proximal muscle weakness (SMA types III and IV). Most patients with SMA have reduced muscle strength and physical dysfunction more or less. Preliminary evidence in people with SMA and in SMA animal models suggests exercise has potential benefits in improving or stabilizing muscle strength and motor function. Physical therapy (PT) in the case with SMA type III to assess the effects.

Methods: MLPA was carried out in a family with maternal consanguineous marriage. We evaluated feasibility, safety, and effects on strength and motor function of a supervised progressive resistance strength training exercise program in the children with SMA types III.

Results: A SMA III pedigree from the Uyghur population was found a homozygous deletion of SMN1 exon 7 and exon 8. Numbers of SMN2 were 4 copies in them. The proband's son is well tolerated to physical training. PT training is beneficial to his physical function and yet improvement in fatigue and muscle strength gets limited.

Conclusions: A two sessions supervised, 3 days/week progressive resistance training exercise program is feasible, safe, and well tolerated in children with SMA III.

1. Introduction

Spinal muscular atrophy (SMA) is one of neuromuscular disorders (NMD), which is resulted from a genetic mutation in the survival motor neuron (SMN) gene with autosomal recessive inheritance (AR) [1]. Individuals with SMA present with observably proximal muscle atrophy and weakness [2, 3]. There are two versions of SMN, SMN1 and SMN2, both located at 5q13.2. This chromosomal region is unstable and liable to gene conversion, deletions, and duplication[4, 5]. SMN1 encodes functional SMN protein, with producing a full-length transcript. Mutations on SMN2 gene, differed from SMN1 by five nucleotides, affect the normal function of the SMN protein[6]. About 94% of SMA patients have a homozygous deletion of SMN1 exon 7. Currently, SMA causing gene is supposed to SMN1 [5, 7]. The SMN2 copy number influences SMA severity [8].

SMA has four clinical types depending on dysfunctional severity and the onset age, from the severest type I to the mildest type IV. The onset of SMA type I occurs prior to six months and those patients fail to sit independently. Patients with SMA type II are able to sit generally yet disabled mobility, the onset between seven months and 18 months of age. Adults with SMA type IV mainly present muscle weakness but remain ambulatory at the age of 30-60 years old[3]. SMA type III (Kugelberg-Welander syndrome) is a

relatively mild subtype, the incidence between the ages of 18 months and 30 years old, showing large clinical heterogeneity. According to the severity of SMA type III phenotype, it can be divided into type IIIa (clinical symptoms before three years of age) and type IIIb (clinical symptoms after three years of age) [9]. Their motor development was usually normal in earlier ages. Most of them reach major motor milestones although the development of walking was delayed in some populations. Muscle weakness mainly occurs at the proximal end, the lower limbs being heavier than the upper limbs. And eventually parts of them lose the ability to walk independently over time. SMA III left half of patients unable to walk during twenty years of life and only a small percentage of them are supposed to remain ambulatory throughout life [3, 10]. Characteristics of SMA III includes the inability to stand up from sitting, to climb stairs unaided, or problems in running and sports [11]. Their life expectancy is not shortened or slightly decreased. Long-term followup studies (follow-up time of two to 20 years) in people with SMA type II and type III suggest a very slow deterioration of muscle strength and motor function [10, 12]. Nevertheless, In general, people with SMA IIIb perform better on functional outcome measures compared to people with SMA type IIIa [13].

The incidence of SMA is about 1/6,000 to 1/10,000 live births, and the carrier frequency is about 1/42 in the Chinese population [14]. Nusinersen is the only disease-modifying therapy for people with SMA, but its benefits in the mildest phenotype are not yet fully known [15]. Previous studies have testified that training exercise programs are beneficial to improvement of muscle strength and motor function [16, 17]. Since SMA is prone to be weakness and disability with age, physical therapy (PT) among SMA patients is suggested [18, 19]. The study aims to the diagnosis of a pedigree and measure the effect of physical exercise training on functional performance in SMA type III.

2. Subjects And Methods

2.1. Subjects and Clinical Evaluation

The study subjects come from a family of Uyghur ethnicity. The boy and his mother presented with progressive muscle weakness and fatigue. The etiology of their myasthenia was unexplained. Both patients were followed by the researcher and routine diagnostic process including metabolic screening (at least amino acids in blood and urine, organic acids in urine and lactate) was negative. And electromyography (EMG) was suggested and a brain magnetic resonance imaging (MRI) scan was clean.

2.2. Physical Therapy Evaluation

Because of no exercise guidelines for SMA patients, measures of rehabilitation are set according to the patient's specific situation. PT programs include cycling on an ergometer, running on a treadmill, and progressive resistance exercise. The training intensity was adjusted according to the patient's debilitated fatigue and work-up of MMT. The trial evaluated the change in functional performance from baseline to two sessions PT, by the grip strength, validated Manual Muscle Testing (MMT; a six-point ordinal scale). Hammersmith Functional Motor Scale - Expanded (HFMSSE), and 6-meter walk test (6MWT). As for the evaluation of MMT, six muscle groups bilaterally were evaluated (shoulder abduction, elbow flexion, wrist

extension, hip flexion, knee extension and ankle dorsiflexion). 6MWT and HFMSE reflect physical performance battery shortly. Weak grip strength is a better indicator of locomotion than low muscle mass. Moreover, care concentrates on fatigue and quality of life. The latter one is assessed with Modified Barthel Index (MBI).

2.3. Multiple ligation-dependent probe amplification (MLPA)

MLPA strategy has been established for detecting duplications and deletions of SMA-related genes[20]. MLPA is a multiplex polymerase chain reaction (PCR) technique with four steps: DNA denaturation and probe hybridization, ligation of probes, PCR of ligated probes, and separation of amplified fragments and data analysis. ①) DNA denaturation and probe hybridization: The sample's DNA was heated at 98 degrees for five minutes. SALSA MLPA P060 SMA probe mixture and MLPA buffer were added to the denaturated sample. The mixing system was heated at 95 degrees for 1 minute and then hybridized at 60 degrees for 16 hours in a warm bath; ②) Ligation of probes: The ligase mixture was added to the hybrid product and was heated at 54 degrees for 15 minutes; ③) PCR of ligated probes: Primers, dNTP and DNA polymerase were added into the mixture in the procedure of 95 degrees 30s, 60 degrees 30s, 72 degrees 60s, 35 cycles; ④) MLPA products were separated and quantified by capillary electrophoresis by an ABI 3130XL Genetic Analyzer with LIZ 500 as the internal size standard. 1ul PCR products were added into mixed liquor of 9ul HIDI and LIZ500. Then the mixture was denatured for 5 minutes in the PCR instrument followed by being cooled to 4 degrees. Then capillary electrophoresis was carried out for about 1 hour. Finally, software Coffalyser.net was used to analyze the results.

3. Results

3.1. Clinical Manifestation

A Chinese pedigree is from the Uyghur population of Xinjiang with consanguineous marriage (Fig. 1). The proband SMA Ⅱ(01-1), the index patient's mother, had onset of illness on 24 years old. Her characteristics are mainly the "duck"-like gait and fatigue. The mother takes good care of herself in daily life. The index patient is diagnosed with SMA Ⅱ(01-2) based on his symmetric muscle weakness in the extremities, chronic progression, good prognosis, and the onset at 15 years of age (in 2018). The proximal decrease in muscle strength was more pronounced. The onset of the disease was manifested as squatting and standing up laboriously, with the help of his hands to support. Gradually he developed to go up stairs by the handrail, run slowly, fall easily. At the age of 16, he suffered from standing independently on one leg, squatting up incompletely and position switching clumsily though he kept ambulatory. Moreover, he presented with weakness of upper-body (bearing 5kg). Physical examination indicated that the adolescent developed muscular atrophy of the limbs, especially the quadriceps femoris and positive Gower signs. Meanwhile, the index patient had vitamin D deficiency (11.8ng/ml, reference:30-100ng/ml). EMG showed multiple progressive neurogenic lesions combined with secondary myogenic lesions in the extremities, heavier in the upper extremities than in the lower extremities and heavier in the proximal extremities than in the distal extremity at the age of 16 years old (Fig. 2).

3.2. Effects of Physical therapy

Summary of findings for the main comparison between pre-treatment and post-treatment for the patient SMA01-2 (Table 1). The HFMSE score increased by 9 points after the PT training (two sessions), indicating clinically meaningful improvement[21]. The training patient showed a slight improvement in muscle strength, as the MMT total score from 21 to 23. The walk function on 6MWT increased by 0.24m/s after PT training. The increase of MBI reflected better quality of life. The trial suggested that training had no significant effects on fatigue. To be noteworthy, the patient had a worse fatigue and increasing trend of creatine kinase in the process of rehabilitation treatment. So the training frequency of 5 times a week was decreased to 3 times a week. After the adjustment, the creatine kinase dropped down compared with before, yet still higher than the normal level. And fatigue did not appear progressive aggravation. Given the economic condition, the patient changed from hospitalization to family care after 2 stages of rehabilitation treatment (about a month). The patient insists on the family-based training mainly by walking and resistance training (resistance training for 20 minutes a day, 3 times a week). His functional status is basically maintained in the 1 year follow-up.

3.3. Mutation detection

Both SMA patients had a homozygous deletion of SMN1 exon 7 and exon 8. Besides, numbers of SMN2 were 4 copies in them. The proband's husband and her daughter were both wild type. The MLPA technique testified that the healthy individual had a heterozygous deletion of SMN1 exon 7 and exon 8. Three copy numbers of gene SMN2 was for the normal function of SMN1 (Fig. 3). Therefore, the inference of a genetic etiology is based on a family history of an AR disorder.

4. Discussion

The study is the first report of a pedigree with SMA of uygur ethnicity. Obvious researches revealed that the proportion of SMA patients with more than 4 SMN2 copies was low in Chinese population[22]. Most of Chinese SMA patients had 2 or 3 SMN2 copies[23]. However, MLPA in this study testified that both patients with SMA3 showed 4 copies of SMN2. In a study of 108 SMA patients and 22 healthy controls, Crawford et al. found that the SMN2 copy number was significantly lower in control subjects[24]. Similar to previous studies, the patient (SMA01-2)'s father carried 3 numbers of SMN2 copy. Therefore, SMA patients appeared to have more SMN2 copy numbers than healthy individuals.

Also, the safety and feasibility of a home-based, supervised, 3 day/week PT exercise programs in adolescent with SMA types IIIb was evaluated. It turns out to be well tolerated in this case. The result was similar to several other studies in different populations. Aga Lewelt et.al reported progressive resistance training was safe in children with SMA types II and III [25]. Biondi et.al performed a progressive running-wheel training program in SMA II-like mice and showed an exercise-induced acceleration of motor-unit maturation at the level of the motor neuron, neuromuscular junction, and muscle fiber, and a delay in

motor neuron death [26]. Measures that supported exercise effectiveness included: grip strength, MMT, HFMSE, and 6MWT. It demonstrates that PT training is beneficial to physical function and yet improvement in fatigue and muscle strength gets limited. To assess effects of physical exercise training on functional performance in people with SMA type Ⅱ and to identify any adverse effects, Bart Bartels et.al dealt with review of literature. The results showed strength and aerobic exercise training is uncertain in people with SMA type Ⅱ. There were several biases that likely influenced the usefulness of this data. Biases included minimal training, lack of blinding, technology malfunction, and variation in time, fatigue, and teenager effort. Moreover, gene copy numbers and gene structures were inferred from MLPA results, so the detailed haplotype of each subjects was unknown. Therefore, further investigations are needed.

5. Conclusions

A deletion mutation of SMN1 gene in exon 7 and 8, which was a homozygotic mutation, was described in a Chinese SMA Ⅱ pedigree. The proband's son with SMA type Ⅱb got improvement in physical function through PT training. And yet improvement in fatigue and muscle strength gets limited.

Abbreviations

SMA	Spinal muscular atrophy
SMN	Survival motor neuron
AR	Autosomal recessive
PT	Physical therapy
MRI	Magnetic resonance imaging
NMD	Neuromuscular disorders
EMG	Electromyography
MLPA	Multiple ligation-dependent probe amplification
MMT	Manual muscle testing
HFMSE	Hammersmith functional motor scale-expanded
6MWT	6-meter walk test
PCR	Polymerase chain reaction

Declarations

Ethics approval and consent to participate The present study was approved by the ethics committee of the People's Hospital of Xinjiang Uygur Autonomous Region (Urumqi, China).

Consent for publication: Informed consent was obtained from the proband and her family.

Availability of supporting data: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: RX and YYW contributed the central idea and analyzed most of the data. JM helped to carried out EMG. CLL, HX, JJC and YPX make contribution to the formulation and implementation of rehabilitation programs. YYW wrote the initial draft of the paper.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

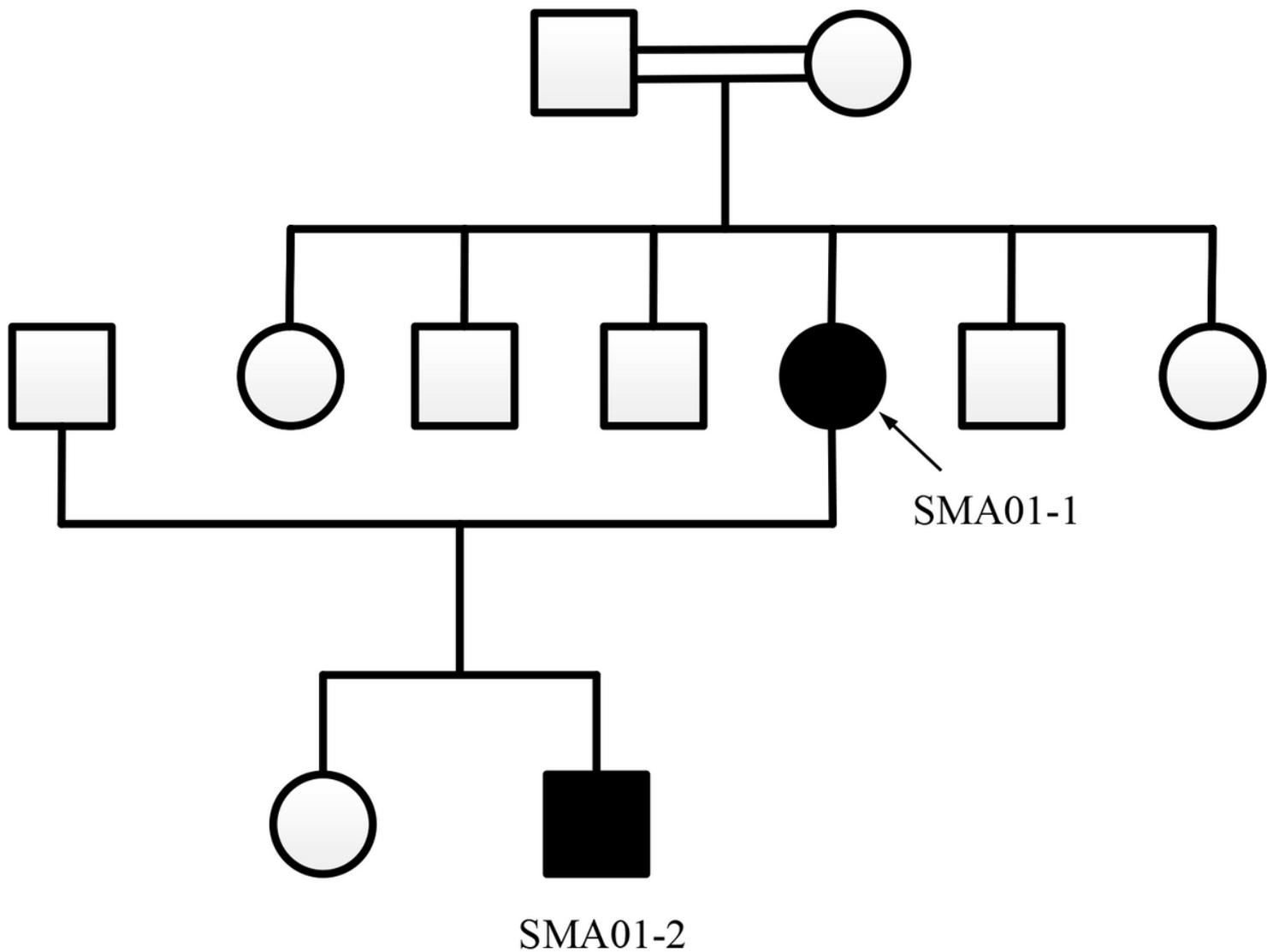


Figure 1

Pedigree of SMA. A square represents a male, and a circle represents female. A double line between a square and a circle represents consanguinity. A shaded symbol shows the affected individual. A SMA01-2 shows the index patient with SMA type Ⅱ. A black arrow shows the proband.

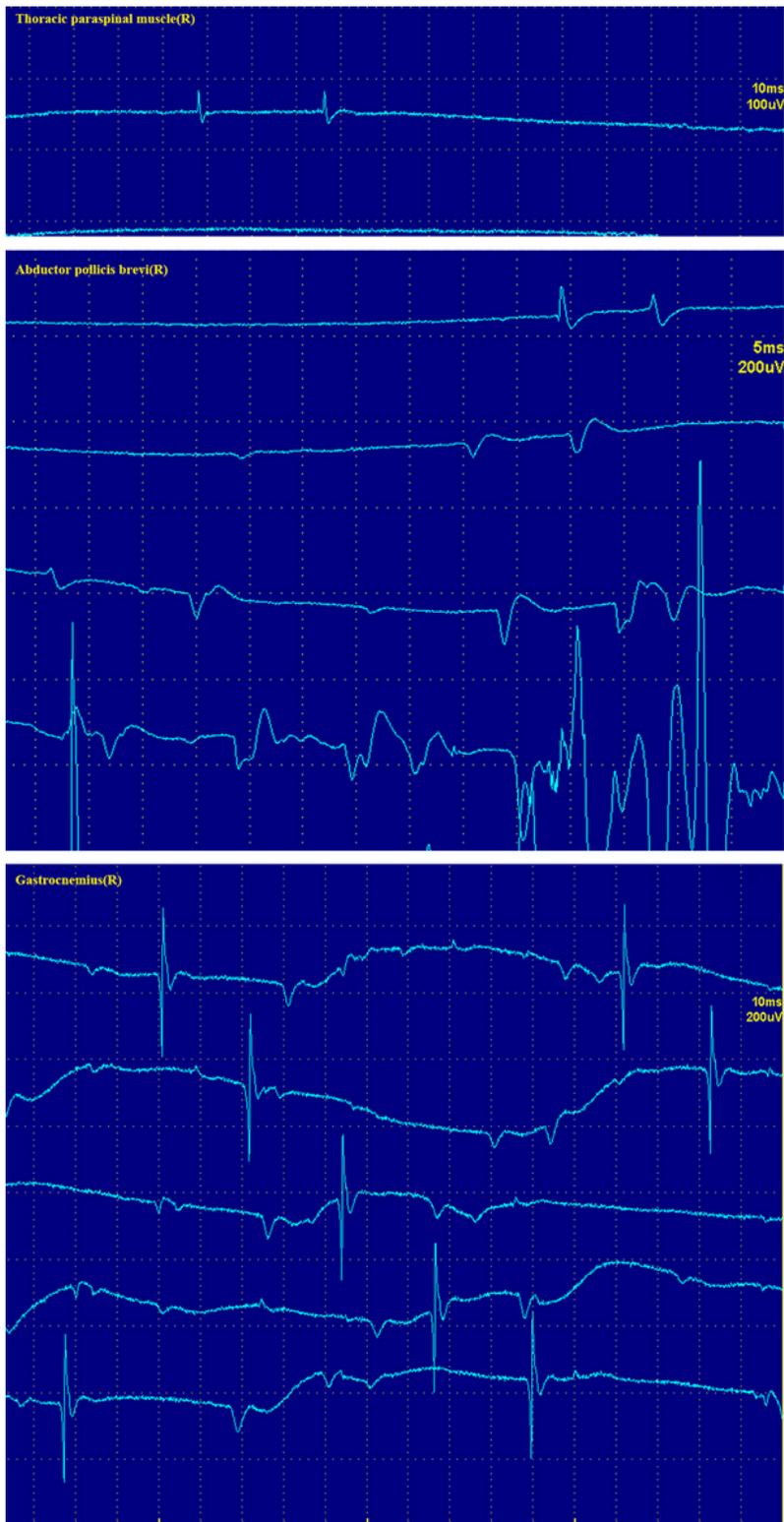
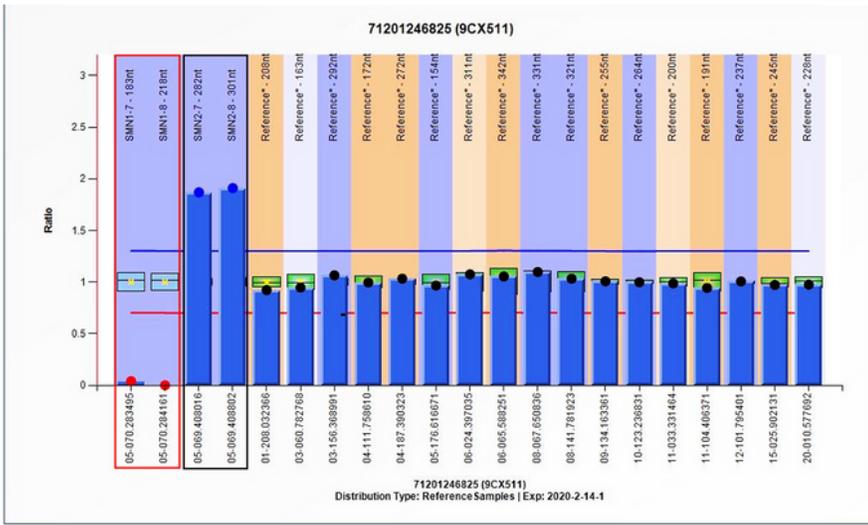
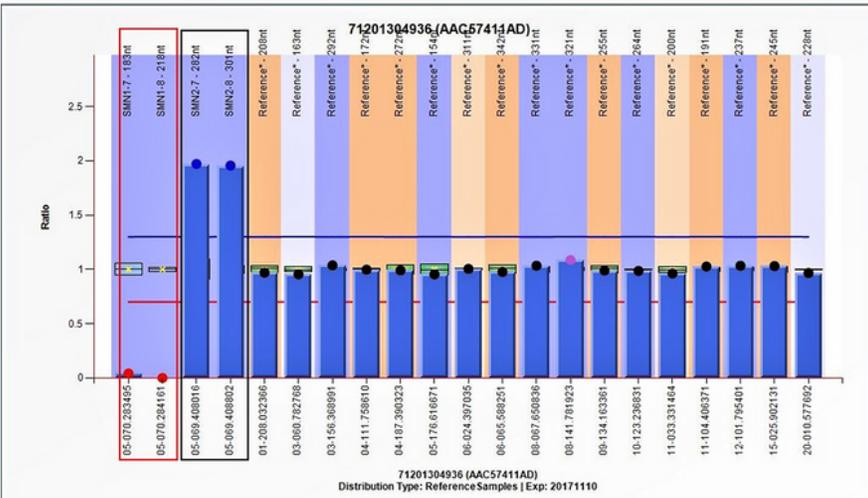


Figure 2

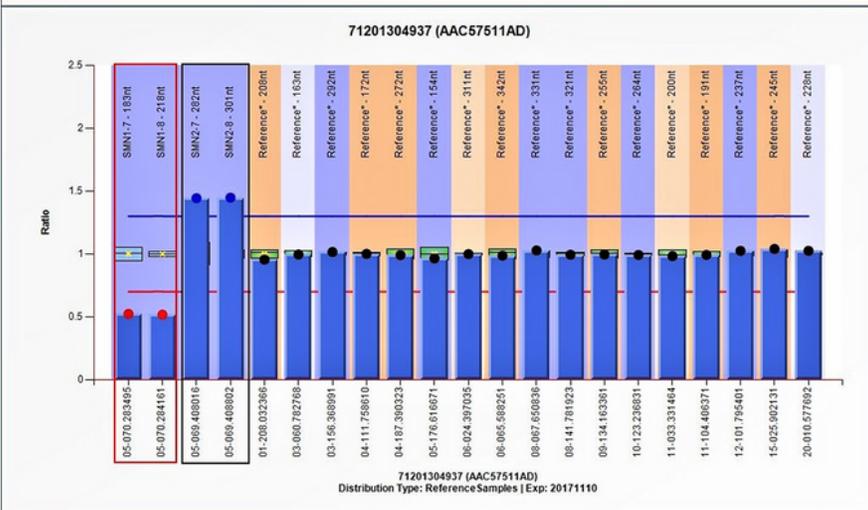
EMG. It shows the presence of thoracic paraspinal muscle, abductor pollicis brevis and gastrocnemius, respectively, on SMA01-2 right limbs.



Index patient



Mother



Father
(wild type)

Figure 3

MLPA. SMN1 deletions and SMN2 copy numbers was founded in SMA01-1 and SMA01-2, comparing to the index patient's father with wild-type.

Supplementary Files

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